TITLE OF THE INVENTION

PROTEIN-PROTEIN INTERACTIONS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] The present application is related to U.S. provisional patent application Serial No. 60/259,571, filed on 4 January 2001, incorporated herein by reference, and claims priority thereto under 35 USC §119(e).

BACKGROUND OF THE INVENTION

[0002] The present invention relates to the discovery of novel protein-protein interactions that are involved in mammalian physiological pathways, including physiological disorders or diseases. Examples of physiological disorders and diseases include non-insulin dependent diabetes mellitus (NIDDM), neurodegenerative disorders, such as Alzheimer's Disease (AD), and the like. Thus, the present invention is directed to complexes of these proteins and/or their fragments, antibodies to the complexes, diagnosis of physiological generative disorders (including diagnosis of a predisposition to and diagnosis of the existence of the disorder), drug screening for agents which modulate the interaction of proteins described herein, and identification of additional proteins in the pathway common to the proteins described herein.

[0003] The publications and other materials used herein to illuminate the background of the invention, and in particular, cases to provide additional details respecting the practice, are incorporated herein by reference, and for convenience, are referenced by author and date in the following text and respectively grouped in the appended Bibliography.

[0004] Many processes in biology, including transcription, translation and metabolic or signal transduction pathways, are mediated by non-covalently associated protein complexes. The formation of protein-protein complexes or protein-DNA complexes produce the most efficient chemical machinery. Much of modern biological research is concerned with identifying proteins involved in cellular processes, determining their functions, and how, when and where they interact with other proteins involved in specific pathways. Further, with rapid advances in genome sequencing, there is a need to define protein linkage maps, i.e., detailed inventories of protein interactions that make up functional assemblies of proteins or protein complexes or that make up physiological pathways.

[0005] Recent advances in human genomics research has led to rapid progress in the identification of novel genes. In applications to biological and pharmaceutical research, there is a

need to determine functions of gene products. A first step in defining the function of a novel gene is to determine its interactions with other gene products in appropriate context. That is, since proteins make specific interactions with other proteins or other biopolymers as part of functional assemblies or physiological pathways, an appropriate way to examine function of a gene is to determine its physical relationship with other genes. Several systems exist for identifying protein interactions and hence relationships between genes.

[0006] There continues to be a need in the art for the discovery of additional protein-protein interactions involved in mammalian physiological pathways. There continues to be a need in the art also to identify the protein-protein interactions that are involved in mammalian physiological disorders and diseases, and to thus identify drug targets.

SUMMARY OF THE INVENTION

[0007] The present invention relates to the discovery of protein-protein interactions that are involved in mammalian physiological pathways, including physiological disorders or diseases, and to the use of this discovery. The identification of the interacting proteins described herein provide new targets for the identification of useful pharmaceuticals, new targets for diagnostic tools in the identification of individuals at risk, sequences for production of transformed cell lines, cellular models and animal models, and new bases for therapeutic intervention in such physiological pathways

[0008] Thus, one aspect of the present invention is protein complexes. The protein complexes are a complex of (a) two interacting proteins, (b) a first interacting protein and a fragment of a second interacting protein, (c) a fragment of a first interacting protein and a second interacting protein, or (d) a fragment of a first interacting protein and a fragment of a second interacting protein. The fragments of the interacting proteins include those parts of the proteins, which interact to form a complex. This aspect of the invention includes the detection of protein interactions and the production of proteins by recombinant techniques. The latter embodiment also includes cloned sequences, vectors, transfected or transformed host cells and transgenic animals.

[0009] A second aspect of the present invention is an antibody that is immunoreactive with the above complex. The antibody may be a polyclonal antibody or a monoclonal antibody. While the antibody is immunoreactive with the complex, it is not immunoreactive with the component parts of the complex. That is, the antibody is not immunoreactive with a first interactive protein, a fragment of a first interacting protein, a second interacting protein or a fragment of a second

interacting protein. Such antibodies can be used to detect the presence or absence of the protein complexes.

[0010] A third aspect of the present invention is a method for diagnosing a predisposition for physiological disorders or diseases in a human or other animal. The diagnosis of such disorders includes a diagnosis of a predisposition to the disorders and a diagnosis for the existence of the disorders. In accordance with this method, the ability of a first interacting protein or fragment thereof to form a complex with a second interacting protein or a fragment thereof is assayed, or the genes encoding interacting proteins are screened for mutations in interacting portions of the protein molecules. The inability of a first interacting protein or fragment thereof to form a complex, or the presence of mutations in a gene within the interacting domain, is indicative of a predisposition to, or existence of a disorder. In accordance with one embodiment of the invention, the ability to form a complex is assayed in a two-hybrid assay. In a first aspect of this embodiment, the ability to form a complex is assayed by a yeast two-hybrid assay. In a second aspect, the ability to form a complex is assayed by a mammalian two-hybrid assay. In a second embodiment, the ability to form a complex is assayed by measuring *in vitro* a complex formed by combining said first protein and said second protein. In one aspect the proteins are isolated from a human or other animal. In a third embodiment, the ability to form a complex is assayed by measuring the binding of an antibody, which is specific for the complex. In a fourth embodiment, the ability to form a complex is assayed by measuring the binding of an antibody that is specific for the complex with a tissue extract from a human or other animal. In a fifth embodiment, coding sequences of the interacting proteins described herein are screened for mutations.

[0011] A fourth aspect of the present invention is a method for screening for drug candidates which are capable of modulating the interaction of a first interacting protein and a second interacting protein. In this method, the amount of the complex formed in the presence of a drug is compared with the amount of the complex formed in the absence of the drug. If the amount of complex formed in the presence of the drug is greater than or less than the amount of complex formed in the absence of the drug, the drug is a candidate for modulating the interaction of the first and second interacting proteins. The drug promotes the interaction if the complex formed in the presence of the drug is greater and inhibits (or disrupts) the interaction if the complex formed in the presence of the drug is less. The drug may affect the interaction directly, i.e., by modulating the binding of the two proteins, or indirectly, e.g., by modulating the expression of one or both of the proteins.

[0012] A fifth aspect of the present invention is a model for such physiological pathways, disorders or diseases. The model may be a cellular model or an animal model, as further described herein. In accordance with one embodiment of the invention, an animal model is prepared by creating transgenic or "knock-out" animals. The knock-out may be a total knock-out, i.e., the desired gene is deleted, or a conditional knock-out, i.e., the gene is active until it is knocked out at a determined time. In a second embodiment, a cell line is derived from such animals for use as a model. In a third embodiment, an animal model is prepared in which the biological activity of a protein complex of the present invention has been altered. In one aspect, the biological activity is altered by disrupting the formation of the protein complex, such as by the binding of an antibody or small molecule to one of the proteins which prevents the formation of the protein complex. In a second aspect, the biological activity of a protein complex is altered by disrupting the action of the complex, such as by the binding of an antibody or small molecule to the protein complex which interferes with the action of the protein complex as described herein. In a fourth embodiment, a cell model is prepared by altering the genome of the cells in a cell line. In one aspect, the genome of the cells is modified to produce at least one protein complex described herein. In a second aspect, the genome of the cells is modified to eliminate at least one protein of the protein complexes described herein.

[0013] A sixth aspect of the present invention are nucleic acids coding for novel proteins discovered in accordance with the present invention and the corresponding proteins and antibodies.

[0014] A seventh aspect of the present invention is a method of screening for drug candidates useful for treating a physiological disorder. In this embodiment, drugs are screened on the basis of the association of a protein with a particular physiological disorder. This association is established in accordance with the present invention by identifying a relationship of the protein with a particular physiological disorder. The drugs are screened by comparing the activity of the protein in the presence and absence of the drug. If a difference in activity is found, then the drug is a drug candidate for the physiological disorder. The activity of the protein can be assayed *in vitro* or *in vivo* using conventional techniques, including transgenic animals and cell lines of the present invention.

DETAILED DESCRIPTION OF THE INVENTION

[0015] The present invention is the discovery of novel interactions between proteins described herein. The genes coding for some of these proteins may have been cloned previously,

but their potential interaction in a physiological pathway or with a particular protein was unknown. Alternatively, the genes coding for some of these proteins have not been cloned previously and represent novel genes. These proteins are identified using the yeast two-hybrid method and searching a human total brain library, as more fully described below.

[0016] According to the present invention, new protein-protein interactions have been discovered. The discovery of these interactions has identified several protein complexes for each protein-protein interaction. The protein complexes for these interactions are set forth below in Tables 1-10, which also identifies the new protein-protein interactions of the present invention.

TABLE 1

Protein Complexes AKT1/FNTA Interaction

Akt1 and alpha subunit of p21 (RAS) farnesyl transferase (FNTA)

A fragment of Akt1 and FNTA

Akt1 and a fragment of FNTA

A fragment of Akt1 and a fragment of FNTA

TABLE 2

Protein Complexes AKT1/TPRD Interaction

Akt1 and tetratricopeptide repeat domain 3 (TPRD)

A fragment of Akt1 and TPRD

Akt1 and a fragment of TPRD

A fragment of Akt1 and a fragment of TPRD

TABLE 3

Protein Complexes AKT1/KIAA0728 Interaction

Akt1 and KIAA0728

A fragment of Akt1 and KIAA0728

Akt1 and a fragment of KIAA0728

A fragment of Akt1 and a fragment of KIAA0728

TABLE 4

Protein Complexes AKT1/PPL Interaction

Akt1 and periplakin (PPL)

A fragment of Akt1 and PPL

Akt1 and a fragment of PPL

A fragment of Akt1 and a fragment of PPL

TABLE 5

Protein Complexes AKT1/Golgin-84 Interaction

Akt1 and Golgin-84

A fragment of Akt1 and Golgin-84

Akt1 and a fragment of Golgin-84

A fragment of Akt1 and a fragment of Golgin-84

TABLE 6

Protein Complexes AKT2/CLIC1 Interaction

Akt2 and chloride intracellular channel protein 1 (CLIC1)

A fragment of Akt2 and CLIC1

Akt2 and a fragment of CLIC1

A fragment of Akt2 and a fragment of CLIC1

TABLE 7

Protein Complexes AKT2/AKR7A2 Interaction

Akt2 and aldo-keto reductase family 7 (AKR7A2)

A fragment of Akt2 and AKR7A2

Akt2 and a fragment of AKR7A2

A fragment of Akt2 and a fragment of AKR7A2

TABLE 8

Protein Complexes AKT2/TPRD Interaction

Akt2 and tetratricopeptide repeat domain 3 (TPRD)

A fragment of Akt2 and TPRD

Akt2 and a fragment of TPRD

A fragment of Akt2 and a fragment of TPRD

TABLE 9

Protein Complexes p90RSK/KIAA0728 Interaction

Ribosomal protein S6 kinase (p90RSK) and KIAA0728

A fragment of p90RSK and KIAA0728

p90RSK and a fragment of KIAA0728

A fragment of p90RSK and a fragment of KIAA0728

TABLE 10

Protein Complexes p90RSK/UNR Interaction

Ribosomal protein S6 kinase (p90RSK) and upstream of N-ras (UNR)

A fragment of p90RSK and UNR

p90RSK and a fragment of UNR

A fragment of p90RSK and a fragment of UNR

[0017] The involvement of above interactions in particular pathways is as follows.

[0018] Many cellular proteins exert their function by interacting with other proteins in the cell. Examples of this are found in the formation of multiprotein complexes and the association of enzymes with their substrates. It is widely believed that a great deal of information can be gained by understanding individual protein-protein interactions, and that this is useful in identifying complex networks of interacting proteins that participate in the workings of normal cellular functions. Ultimately, the knowledge gained by characterizing these networks can lead to valuable insight into the causes of human diseases and can eventually lead to the development of therapeutic strategies. The yeast two-hybrid assay is a powerful tool for determining protein-protein interactions and it has been successfully used for studying human disease pathways. In one variation of this technique, a protein of interest (or a portion of that protein) is expressed in a population of yeast

cells that collectively contain all protein sequences. Yeast cells that possess protein sequences that interact with the protein of interest are then genetically selected, and the identity of those interacting proteins are determined by DNA sequencing. Thus, proteins that can be demonstrated to interact with a protein known to be involved in a human disease are therefore also implicated in that disease. Proteins identified in the first round of two-hybrid screening can be subsequently used in a second round of two-hybrid screening, allowing the identification of multiple proteins in the complex network of interactions in a disease pathway.

[0019] Akt1 and Akt2 are serine/threonine protein kinases capable of phosphorylating a variety of known proteins. Akt1 and Akt2 are activated by platelet-derived growth factor (PDGF), a growth factor involved in the decision between cellular proliferation and apoptosis (Franke et al., 1995). AKT kinases are also activated by insulin-like growth factor (IGF1), and in this capacity are involved in survival of cerebellar neurons (Dudek et al., 1997). Furthermore, Akt1 is involved in the activation of NFkB by tumor necrosis factor (TNF) (Ozes et al., 1999). Akt2 has been shown to be associated with pancreatic carcinomas (Cheng et al., 1996). Akt kinases have been implicated in insulin-regulated glucose transport and the development of non-insulin dependent diabetes mellitus (Krook et al., 1998).

[0020] The p90/RSK kinase (also known as HU1) is also involved in intracellular signaling cascades relevant to human disease. p90/RSK activity is regulated by growth factors, and the phosphorylation of two p90/RSK substrates, BAD and CREB, suppresses apoptosis in neurons (Bonni et al., 1999). p90/RSK is also implicated in cell cycle control in response to Mos-MEK1 signaling (Bhatt and Ferrell, 1999; Gross et al., 1999).

[0021] Clearly, these kinases play varied and important roles in a number of intracellular signaling pathways, and are thus good starting points from which to identify novel protein interactions that define disease-related signal transduction pathways. To this end, Akt1 and Akt2 were used in yeast two-hybrid assays to identify Akt-interacting proteins that may be potential targets for drug intervention. Here, we describe new protein-protein interactions for Akt1, Akt2, and p90/RSK.

[0022] The first interactor for Akt1 is the alpha subunit of p21 (RAS) farnesyl transferase (FNTA). FNTA has been shown to bind to both the TGF-beta and activin receptors in the yeast two-hybrid assay (Ventura et al., 1996; Wang et al., 1996). Further, it has been shown that FNTA binds to the TGF-beta receptor in the absence of ligand, and that ligand binding causes the phosphorylation and release of FNTA. Presumably, FNTA is then free to interact with other

cytoplasmic factors in the transmission of the TGF-beta signal. The finding that Akt1 interacts with FNTA suggests a direct connection between receptors at the cell surface and the intracellular signal transduction machinery involving Akt1.

[0023] The second interactor for Akt1 is the periplakin protein. The plakins are cytoskeletal coiled-coil proteins that bind to intermediate filaments as well as actin and microtubule networks. Periplakin has been shown to bind to the intracellular portion of collagen type XVII in a yeast two-hybrid assay (Aho et al., 1998). Periplakin appears to be highly expressed in tissues that are rich in epithelial cells. The interaction of periplakin with Akt1 suggests it may be a substrate of this kinase, and that its function may be modulated by phosphorylation. Alternatively, the subcellular localization of Akt1 may be altered by its interaction with periplakin.

[0024] The hypothetical protein KIAA0728 was found as an interactor of both Akt1 and p90/RSK. KIAA0728 contains an EF hand calcium-binding motif, a nuclear localization sequence and six spectrin repeats. The Akt1- and p90/RSK-interacting regions of KIAA0728 overlap, suggesting these proteins may bind the same domain of KIAA0728. The interaction of KIAA0728 with both Akt1 and p90/RSK suggests that it may act as a substrate for both enzymes, or alternatively that KIAA0728, by virtue of its spectrin repeats, may serve as a scaffold to link these two kinases together.

[0025] Akt1 is found to interact with the integral membrane protein Golgin-84. Golgin-84 is a coiled-coil containing protein that was originally isolated as a yeast two-hybrid interactor of the OCRL1 phosphatidylinositol(4,5)P2 5-phosphatase that is implicated in oculocerebrorenal syndrome (Bascom et al., 1999). *In vitro* studies indicate that most of the golgin-84 protein is predicted to be cytoplasmic with only the most extreme C-terminus of the protein extending to the extracellular/vesicular side of membranes. Not surprisingly, the cytoplasmic portion of golgin-84 associates with Akt1.

[0026] The TPR domain protein TPRD was found to interact with both Akt1 and Akt2. TPDR may play a major role in development since it is localized to the Down syndrome-critical region on human chromosome 21q22.2 (Ohira et al., 1996; Tsukahara et al., 1996). Analysis of the amino acid sequence of TPRD reveals the presence of TPR repeats towards the N-terminus of the protein, a bipartite nuclear localization sequence, and a zinc finger. The region of TPRD that associates with the two Akts (amino acids 1058 to 1189) is located near the center of the protein and is distinct from any of the predicted structural domains.

[0027] Akt2 is found to interact with the aldehyde reductase AKR7A2 (aflatoxin B1-dialdehyde reductase or AFAR). AKR7A2 is an aldoketoreductase that resides in the cytoplasm of many if not all tissues. AKR7A2 appears to be highly regulated at the transcriptional level. Studies using rats have demonstrated that AKR7A2 mRNA and protein levels increase dramatically in the liver following exposure to dietary antioxidants (Ellis et al., 1996). The finding that AKR7A2 associates with Akt2 suggests that perhaps this enzyme is also regulated at the post-translational level by Akt2.

[0028] The intracellular chloride channel protein CLIC1 was shown to interact with Akt2. CLIC1, also known as NCC27 (nuclear chloride channel-27), was first cloned from human U937 myelomonocytic cells and is the first member of the CLIC family of chloride channels (Valenzuela et al., 1997). CLIC1 primarily localizes to the nuclear membrane and likely plays a role in the transport of chloride into the nucleus. The finding that CLIC1 and Akt2 associate with one another is rather intriguing, and it suggests that Akt2 may play a role in regulating nuclear ion transport. Interestingly, another related CLIC family member that localizes to the nuclear membrane, CLIC3, has been demonstrated to interact with a signal transduction protein, ERK7 (Qian et al., 1999). Taken together, these results suggest that intracellular chloride channels may be intimately linked to transduction of extracellular signals.

[0029] Finally, the UNR (upstream of N-ras) protein was shown to associate with p90/RSK. UNR has no known function though it does contain several cold shock DNA-binding domains and two predicted peroxidase active sites. Transcription of UNR, which is located immediately upstream of the N-ras gene, interferes with transcription of N-ras (Boussadia et al., 1997). Furthermore, the human and rat UNR genes appear to undergo exon skipping that is tissue-dependent (Boussadia et al., 1993). Interestingly, one of the UNR protein products has been shown to interact with the protein product of the ALL-1 gene, which is involved in human chromosome translocations and other rearrangements in acute lymphocytic leukemia (Leshkowitz et al., 1996). ALL-1 is the human homolog of the Drosophila trithorax protein and plays a role in the regulation of homeotic genes involved in body segmentation. The finding that p90/RSK binds to UNR suggests that RSK may be capable of phosphorylating UNR, thereby affecting its function. Because UNR interacts with ALL-1, it seems likely that such regulation of UNR by p90/RSK might affect gene transcription.

[0030] The proteins disclosed in the present invention were found to interact with their corresponding proteins in the yeast two-hybrid system. Because of the involvement of the corresponding proteins in the physiological pathways disclosed herein, the proteins disclosed herein also participate in the same physiological pathways. Therefore, the present invention provides a list of uses of these proteins and DNA encoding these proteins for the development of diagnostic and therapeutic tools useful in the physiological pathways. This list includes, but is not limited to, the following examples.

Two-Hybrid System

[0031] The principles and methods of the yeast two-hybrid system have been described in detail elsewhere (e.g., Bartel and Fields, 1997; Bartel et al., 1993; Fields and Song, 1989; Chevray and Nathans, 1992). The following is a description of the use of this system to identify proteins that interact with a protein of interest.

[0032] The target protein is expressed in yeast as a fusion to the DNA-binding domain of the yeast Gal4p. DNA encoding the target protein or a fragment of this protein is amplified from cDNA by PCR or prepared from an available clone. The resulting DNA fragment is cloned by ligation or recombination into a DNA-binding domain vector (e.g., pGBT9, pGBT.C, pAS2-1) such that an in-frame fusion between the Gal4p and target protein sequences is created.

[0033] The target gene construct is introduced, by transformation, into a haploid yeast strain. A library of activation domain fusions (i.e., adult brain cDNA cloned into an activation domain vector) is introduced by transformation into a haploid yeast strain of the opposite mating type. The yeast strain that carries the activation domain constructs contains one or more Gal4p-responsive reporter gene(s), whose expression can be monitored. Examples of some yeast reporter strains include Y190, PJ69, and CBY14a. An aliquot of yeast carrying the target gene construct is combined with an aliquot of yeast carrying the activation domain library. The two yeast strains mate to form diploid yeast and are plated on media that selects for expression of one or more Gal4p-responsive reporter genes. Colonies that arise after incubation are selected for further characterization.

[0034] The activation domain plasmid is isolated from each colony obtained in the two-hybrid search. The sequence of the insert in this construct is obtained by the dideoxy nucleotide chain termination method. Sequence information is used to identify the gene/protein encoded by the activation domain insert via analysis of the public nucleotide and protein databases. Interaction of the activation domain fusion with the target protein is confirmed by testing for the specificity of the

interaction. The activation domain construct is co-transformed into a yeast reporter strain with either the original target protein construct or a variety of other DNA-binding domain constructs. Expression of the reporter genes in the presence of the target protein but not with other test proteins indicates that the interaction is genuine.

[0035] In addition to the yeast two-hybrid system, other genetic methodologies are available for the discovery or detection of protein-protein interactions. For example, a mammalian two-hybrid system is available commercially (Clontech, Inc.) that operates on the same principle as the yeast two-hybrid system. Instead of transforming a yeast reporter strain, plasmids encoding DNA-binding and activation domain fusions are transfected along with an appropriate reporter gene (e.g., lacZ) into a mammalian tissue culture cell line. Because transcription factors such as the *Saccharomyces cerevisiae* Gal4p are functional in a variety of different eukaryotic cell types, it would be expected that a two-hybrid assay could be performed in virtually any cell line of eukaryotic origin (e.g., insect cells (SF9), fungal cells, worm cells, etc.). Other genetic systems for the detection of protein-protein interactions include the so-called SOS recruitment system (Aronheim et al., 1997).

Protein-protein interactions

[0036] Protein interactions are detected in various systems including the yeast two-hybrid system, affinity chromatography, co-immunoprecipitation, subcellular fractionation and isolation of large molecular complexes. Each of these methods is well characterized and can be readily performed by one skilled in the art. See, e.g., U.S. Patents No. 5,622,852 and 5,773,218, and PCT published applications No. WO 97/27296 and WO 99/65939, each of which are incorporated herein by reference.

[0037] The protein of interest can be produced in eukaryotic or prokaryotic systems. A cDNA encoding the desired protein is introduced in an appropriate expression vector and transfected in a host cell (which could be bacteria, yeast cells, insect cells, or mammalian cells). Purification of the expressed protein is achieved by conventional biochemical and immunochemical methods well known to those skilled in the art. The purified protein is then used for affinity chromatography studies: it is immobilized on a matrix and loaded on a column. Extracts from cultured cells or homogenized tissue samples are then loaded on the column in appropriate buffer, and non-binding proteins are eluted. After extensive washing, binding proteins or protein complexes are eluted using various methods such as a gradient of pH or a gradient of salt concentration. Eluted proteins can then be separated by two-dimensional gel electrophoresis, eluted from the gel, and identified by

micro-sequencing. The purified proteins can also be used for affinity chromatography to purify interacting proteins disclosed herein. All of these methods are well known to those skilled in the art.

[0038] Similarly, both proteins of the complex of interest (or interacting domains thereof) can be produced in eukaryotic or prokaryotic systems. The proteins (or interacting domains) can be under control of separate promoters or can be produced as a fusion protein. The fusion protein may include a peptide linker between the proteins (or interacting domains) which, in one embodiment, serves to promote the interaction of the proteins (or interacting domains). All of these methods are also well known to those skilled in the art.

[0039] Purified proteins of interest, individually or a complex, can also be used to generate antibodies in rabbit, mouse, rat, chicken, goat, sheep, pig, guinea pig, bovine, and horse. The methods used for antibody generation and characterization are well known to those skilled in the art. Monoclonal antibodies are also generated by conventional techniques. Single chain antibodies are further produced by conventional techniques.

[0040] DNA molecules encoding proteins of interest can be inserted in the appropriate expression vector and used for transfection of eukaryotic cells such as bacteria, yeast, insect cells, or mammalian cells, following methods well known to those skilled in the art. Transfected cells expressing both proteins of interest are then lysed in appropriate conditions, one of the two proteins is immunoprecipitated using a specific antibody, and analyzed by polyacrylamide gel electrophoresis. The presence of the binding protein (co-immunoprecipitated) is detected by immunoblotting using an antibody directed against the other protein. Co-immunoprecipitation is a method well known to those skilled in the art.

[0041] Transfected eukaryotic cells or biological tissue samples can be homogenized and fractionated in appropriate conditions that will separate the different cellular components. Typically, cell lysates are run on sucrose gradients, or other materials that will separate cellular components based on size and density. Subcellular fractions are analyzed for the presence of proteins of interest with appropriate antibodies, using immunoblotting or immunoprecipitation methods. These methods are all well known to those skilled in the art.

Disruption of protein-protein interactions

[0042] It is conceivable that agents that disrupt protein-protein interactions can be beneficial in many physiological disorders, including, but not-limited to NIDDM, AD and others disclosed

herein. Each of the methods described above for the detection of a positive protein-protein interaction can also be used to identify drugs that will disrupt said interaction. As an example, cells transfected with DNAs coding for proteins of interest can be treated with various drugs, and co-immunoprecipitations can be performed. Alternatively, a derivative of the yeast two-hybrid system, called the reverse yeast two-hybrid system (Leanna and Hannink, 1996), can be used, provided that the two proteins interact in the straight yeast two-hybrid system.

Modulation of protein-protein interactions

[0043] Since the interactions described herein are involved in a physiological pathway, the identification of agents which are capable of modulating the interactions will provide agents which can be used to track physiological disorder or to use lead compounds for development of therapeutic agents. An agent may modulate expression of the genes of interacting proteins, thus affecting interaction of the proteins. Alternatively, the agent may modulate the interaction of the proteins. The agent may modulate the interaction of wild-type with wild-type proteins, wild-type with mutant proteins, or mutant with mutant proteins. Agents which may be used to modulate the protein interaction inlcude a peptide, an antibody, a nucleic acid, an antisense compound or a ribozyme. The nucleic acid may encode the antibody or the antisense compound. The peptide may be at least 4 amino acids of the sequence of either of the interacting proteins. Alternatively, the peptide may be from 4 to 30 amino acids (or from 8 to 20 amino acids) that is at least 75% identical to a contiguous span of amino acids of either of the interacting proteins. The peptide may be covalently linked to a transporter capable of increasing cellular uptake of the peptide. Examples of a suitable transporter include penetratins, *l*-Tat_{49-57}, *d*-Tat_{49-57}, retro-inverso isomers of *l*- or *d*-Tat_{49-57}, L-arginine oligomers, D- arginine oligomers, L-lysine oligomers, D-lysine oligomers, L-histine oligomers, D-histine oligomers, L-ornithine oligomers, D-ornithine oligomers, short peptide sequences derived from fibroblast growth factor, Galparan, and HSV-1 structural protein VP22, and peptoid analogs thereof. Agents can be tested using transfected host cells, cell lines, cell models or animals, such as described herein, by techniques well known to those of ordinary skill in the art, such as disclosed in U.S. Patents Nos. 5,622,852 and 5,773,218, and PCT published application Nos. WO 97/27296 and WO 99/65939, each of which are incorporated herein by reference. The modulating effect of the agent can be tested *in vivo* or *in vitro*. Agents can be provided for testing in a phage display library or a combinatorial library. Exemplary of a method to screen agents is to measure the effect that the agent has on the formation of the protein complex.

Mutation screening

[0044] The proteins disclosed in the present invention interact with one or more proteins known to be involved in a physiological pathway, such as in NIDDM, AD or pathways described herein. Mutations in interacting proteins could also be involved in the development of the physiological disorder, such as NIDDM, AD or disorders described herein, for example, through a modification of protein-protein interaction, or a modification of enzymatic activity, modification of receptor activity, or through an unknown mechanism. Therefore, mutations can be found by sequencing the genes for the proteins of interest in patients having the physiological disorder, such as insulin, and non-affected controls. A mutation in these genes, especially in that portion of the gene involved in protein interactions in the physiological pathway, can be used as a diagnostic tool and the mechanistic understanding the mutation provides can help develop a therapeutic tool.

Screening for at-risk individuals

[0045] Individuals can be screened to identify those at risk by screening for mutations in the protein disclosed herein and identified as described above. Alternatively, individuals can be screened by analyzing the ability of the proteins of said individual disclosed herein to form natural complexes. Further, individuals can be screened by analyzing the levels of the complexes or individual proteins of the complexes or the mRNA encoding the protein members of the complexes. Techniques to detect the formation of complexes, including those described above, are known to those skilled in the art. Techniques and methods to detect mutations are well known to those skilled in the art. Techniques to detect the level of the complexes, proteins or mRNA are well known to those skilled in the art.

Cellular models of Physiological Disorders

[0046] A number of cellular models of many physiological disorders or diseases have been generated. The presence and the use of these models are familiar to those skilled in the art. As an example, primary cell cultures or established cell lines can be transfected with expression vectors encoding the proteins of interest, either wild-type proteins or mutant proteins. The effect of the proteins disclosed herein on parameters relevant to their particular physiological disorder or disease can be readily measured. Furthermore, these cellular systems can be used to screen drugs that will influence those parameters, and thus be potential therapeutic tools for the particular physiological

disorder or disease. Alternatively, instead of transfecting the DNA encoding the protein of interest, the purified protein of interest can be added to the culture medium of the cells under examination, and the relevant parameters measured.

Animal models

[0047] The DNA encoding the protein of interest can be used to create animals that overexpress said protein, with wild-type or mutant sequences (such animals are referred to as "transgenic"), or animals which do not express the native gene but express the gene of a second animal (referred to as "transplacement"), or animals that do not express said protein (referred to as "knock-out"). The knock-out animal may be an animal in which the gene is knocked out at a determined time. The generation of transgenic, transplacement and knock-out animals (normal and conditioned) uses methods well known to those skilled in the art.

[0048] In these animals, parameters relevant to the particular physiological disorder can be measured. These parametes may include receptor function, protein secretion *in vivo* or *in vitro*, survival rate of cultured cells, concentration of particular protein in tissue homogenates, signal transduction, behavioral analysis, protein synthesis, cell cycle regulation, transport of compounds across cell or nuclear membranes, enzyme activity, oxidative stress, production of pathological products, and the like. The measurements of biochemical and pathological parameters, and of behavioral parameters, where appropriate, are performed using methods well known to those skilled in the art. These transgenic, transplacement and knock-out animals can also be used to screen drugs that may influence the biochemical, pathological, and behavioral parameters relevant to the particular physiological disorder being studied. Cell lines can also be derived from these animals for use as cellular models of the physiological disorder, or in drug screening.

Rational drug design

[0049] The goal of rational drug design is to produce structural analogs of biologically active polypeptides of interest or of small molecules with which they interact (e.g., agonists, antagonists, inhibitors) in order to fashion drugs which are, for example, more active or stable forms of the polypeptide, or which, e.g., enhance or interfere with the function of a polypeptide *in vivo*. Several approaches for use in rational drug design include analysis of three-dimensional structure, alanine scans, molecular modeling and use of anti-id antibodies. These techniques are well known to those skilled in the art. Such techniques may include providing atomic coordinates defining a

three-dimensional structure of a protein complex formed by said first polypeptide and said second polypeptide, and designing or selecting compounds capable of interfering with the interaction between a first polypeptide and a second polypeptide based on said atomic coordinates.

[0050] Following identification of a substance which modulates or affects polypeptide activity, the substance may be further investigated. Furthermore, it may be manufactured and/or used in preparation, i.e., manufacture or formulation, or a composition such as a medicament, pharmaceutical composition or drug. These may be administered to individuals.

[0051] A substance identified as a modulator of polypeptide function may be peptide or non-peptide in nature. Non-peptide "small molecules" are often preferred for many *in vivo* pharmaceutical uses. Accordingly, a mimetic or mimic of the substance (particularly if a peptide) may be designed for pharmaceutical use.

[0052] The designing of mimetics to a known pharmaceutically active compound is a known approach to the development of pharmaceuticals based on a "lead" compound. This approach might be desirable where the active compound is difficult or expensive to synthesize or where it is unsuitable for a particular method of administration, e.g., pure peptides are unsuitable active agents for oral compositions as they tend to be quickly degraded by proteases in the alimentary canal. Mimetic design, synthesis and testing is generally used to avoid randomly screening large numbers of molecules for a target property.

[0053] Once the pharmacophore has been found, its structure is modeled according to its physical properties, e.g., stereochemistry, bonding, size and/or charge, using data from a range of sources, e.g., spectroscopic techniques, x-ray diffraction data and NMR. Computational analysis, similarity mapping (which models the charge and/or volume of a pharmacophore, rather than the bonding between atoms) and other techniques can be used in this modeling process.

[0054] A template molecule is then selected, onto which chemical groups that mimic the pharmacophore can be grafted. The template molecule and the chemical groups grafted thereon can be conveniently selected so that the mimetic is easy to synthesize, is likely to be pharmacologically acceptable, and does not degrade *in vivo*, while retaining the biological activity of the lead compound. Alternatively, where the mimetic is peptide-based, further stability can be achieved by cyclizing the peptide, increasing its rigidity. The mimetic or mimetics found by this approach can then be screened to see whether they have the target property, or to what extent it is exhibited. Further optimization or modification can then be carried out to arrive at one or more final mimetics for *in vivo* or clinical testing.

Diagnostic Assays

[0055] The identification of the interactions disclosed herein enables the development of diagnostic assays and kits, which can be used to determine a predisposition to or the existence of a physiological disorder. In one aspect, one of the proteins of the interaction is used to detect the presence of a "normal" second protein (i.e., normal with respect to its ability to interact with the first protein) in a cell extract or a biological fluid, and further, if desired, to detect the quantitative level of the second protein in the extract or biological fluid. The absence of the "normal" second protein would be indicative of a predisposition or existence of the physiological disorder. In a second aspect, an antibody against the protein complex is used to detect the presence and/or quantitative level of the protein complex. The absence of the protein complex would be indicative of a predisposition or existence of the physiological disorder.

Nucleic Acids and Proteins

[0056] A nucleic acid or fragment thereof has substantial identity with another if, when optimally aligned (with appropriate nucleotide insertions or deletions) with the other nucleic acid (or its complementary strand), there is nucleotide sequence identity in at least about 60% of the nucleotide bases, usually at least about 70%, more usually at least about 80%, preferably at least about 90%, more preferably at least about 95% of the nucleotide bases, and more preferably at least about 98% of the nucleotide bases. A protein or fragment thereof has substantial identity with another if, optimally aligned, there is an amino acid sequence identity of at least about 30% identity with an entire naturally-occurring protein or a portion thereof, usually at least about 70% identity, more ususally at least about 80% identity, preferably at least about 90% identity, more preferably at least about 95% identity, and most preferably at least about 98% identity.

[0057] Identity means the degree of sequence relatedness between two polypeptide or two polynucleotides sequences as determined by the identity of the match between two strings of such sequences. Identity can be readily calculated. While there exist a number of methods to measure identity between two polynucleotide or polypeptide sequences, the term "identity" is well known to skilled artisans (*Computational Molecular Biology*, Lesk, A. M., ed., Oxford University Press, New York, 1988; *Biocomputing: Informatics and Genome Projects*, Smith, D. W., ed., Academic Press, New York, 1993; *Computer Analysis of Sequence Data*, Part I, Griffin, A. M., and Griffin, H. G., eds., Humana Press, New Jersey, 1994; *Sequence Analysis in Molecular Biology*, von Heinje,

G., Academic Press, 1987; and *Sequence Analysis Primer*, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991). Methods commonly employed to determine identity between two sequences include, but are not limited to those disclosed in *Guide to Huge Computers*, Martin J. Bishop, ed., Academic Press, San Diego, 1994, and Carillo, H., and Lipman, D., *SIAM J Applied Math.* **48**:1073 (1988). Preferred methods to determine identity are designed to give the largest match between the two sequences tested. Such methods are codified in computer programs. Preferred computer program methods to determine identity between two sequences include, but are not limited to, GCG (Genetics Computer Group, Madison Wis.) program package (Devereux, J., et al., *Nucleic Acids Research* **12**(1).387 (1984)), BLASTP, BLASTN, FASTA (Altschul et al. (1990); Altschul et al. (1997)). The well-known Smith Waterman algorithm may also be used to determine identity.

[0058] Alternatively, substantial homology or similarity exists when a nucleic acid or fragment thereof will hybridize to another nucleic acid (or a complementary strand thereof) under selective hybridization conditions, to a strand, or to its complement. Selectivity of hybridization exists when hybridization which is substantially more selective than total lack of specificity occurs. Nucleic acid hybridization will be affected by such conditions as salt concentration, temperature, or organic solvents, in addition to the base composition, length of the complementary strands, and the number of nucleotide base mismatches between the hybridizing nucleic acids, as will be readily appreciated by those skilled in the art. Stringent temperature conditions will generally include temperatures in excess of 30^{o}C, typically in excess of 37^{o}C, and preferably in excess of 45^{o}C. Stringent salt conditions will ordinarily be less than 1000 mM, typically less than 500 mM, and preferably less than 200 mM. However, the combination of parameters is much more important than the measure of any single parameter. See, e.g., Asubel, 1992; Wetmur and Davidson, 1968.

[0059] The terms "isolated", "substantially pure", and "substantially homogeneous" are used interchangeably to describe a protein or polypeptide which has been separated from components which accompany it in its natural state. A monomeric protein is substantially pure when at least about 60 to 75% of a sample exhibits a single polypeptide sequence. A substantially pure protein will typically comprise about 60 to 90% W/W of a protein sample, more usually about 95%, and preferably will be over about 99% pure. Protein purity or homogeneity may be indicated by a number of means well known in the art, such as polyacrylamide gel electrophoresis of a protein sample, followed by visualizing a single polypeptide band upon staining the gel. For certain

purposes, higher resolution may be provided by using HPLC or other means well known in the art which are utilized for purification.

[0060] Large amounts of the nucleic acids of the present invention may be produced by (a) replication in a suitable host or transgenic animals or (b) chemical synthesis using techniques well known in the art. Constructs prepared for introduction into a prokaryotic or eukaryotic host may comprise a replication system recognized by the host, including the intended polynucleotide fragment encoding the desired polypeptide, and will preferably also include transcription and translational initiation regulatory sequences operably linked to the polypeptide encoding segment. Expression vectors may include, for example, an origin of replication or autonomously replicating sequence (ARS) and expression control sequences, a promoter, an enhancer and necessary processing information sites, such as ribosome-binding sites, RNA splice sites, polyadenylation sites, transcriptional terminator sequences, and mRNA stabilizing sequences. Secretion signals may also be included where appropriate which allow the protein to cross and/or lodge in cell membranes, and thus attain its functional topology, or be secreted from the cell. Such vectors may be prepared by means of standard recombinant techniques well known in the art.

[0061] The nucleic acid or protein may also be incorporated on a microarray. The preparation and use of microarrays are well known in the art. Generally, the microarray may contain the entire nucleic acid or protein, or it may contain one or more fragments of the nucleic acid or protein. Suitable nucleic acid fragments may include at least 17 nucleotides, at least 21 nucleotides, at least 30 nucleotides or at least 50 nucleotides of the nucleic acid sequence, particularly the coding sequence. Suitable protein fragments may include at least 4 amino acids, at least 8 amino acids, at least 12 amino acids, at least 15 amino acids, at least 17 amino acids or at least 20 amino acids. Thus, the present invention is also directed to such nucleic acid and protein fragments.

EXAMPLES

[0062] The present invention is further detailed in the following Examples, which are offered by way of illustration and are not intended to limit the invention in any manner. Standard techniques well known in the art or the techniques specifically described below are utilized.

EXAMPLE 1

Yeast Two-Hybrid System

[0063] The principles and methods of the yeast two-hybrid systems have been described in detail (Bartel and Fields, 1997). The following is thus a description of the particular procedure that we used, which was applied to all proteins.

[0064] The cDNA encoding the bait protein was generated by PCR from brain cDNA. Gene-specific primers were synthesized with appropriate tails added at their 5' ends to allow recombination into the vector pGBTQ. The tail for the forward primer was 5'-GCAGGAAACAGCTATGACCATACAGTCAGCGGCCGCCACC-3' (SEQ ID NO:1) and the tail for the reverse primer was 5'-ACGGCCAGTCGCGTGGAGTGTTATGTCATGCGGCCGCTA-3' (SEQ ID NO:2). The tailed PCR product was then introduced by recombination into the yeast expression vector pGBTQ, which is a close derivative of pGBTC (Bartel et al., 1996) in which the polylinker site has been modified to include M13 sequencing sites. The new construct was selected directly in the yeast J693 for its ability to drive tryptophane synthesis (genotype of this strain: Mat α, ade2, his3, leu2, trp1, URA3::GAL1-lacZ LYS2::GAL1-HIS3 gal4del gal80del cyhR2). In these yeast cells, the bait is produced as a C-terminal fusion protein with the DNA binding domain of the transcription factor Gal4 (amino acids 1 to 147). A total human brain (37 year-old male Caucasian) cDNA library cloned into the yeast expression vector pACT2 was purchased from Clontech (human brain MATCHMAKER cDNA, cat. # HL4004AH), transformed into the yeast strain J692 (genotype of this strain: Mat a, ade2, his3, leu2, trp1, URA3::GAL1-lacZ LYS2::GAL1-HIS3 gal4del gal80del cyhR2), and selected for the ability to drive leucine synthesis. In these yeast cells, each cDNA is expressed as a fusion protein with the transcription activation domain of the transcription factor Gal4 (amino acids 768 to 881) and a 9 amino acid hemagglutinin epitope tag. J693 cells (Mat α type) expressing the bait were then mated with J692 cells (Mat a type) expressing proteins from the brain library. The resulting diploid yeast cells expressing proteins interacting with the bait protein were selected for the ability to synthesize tryptophan, leucine, histidine, and β-galactosidase. DNA was prepared from each clone, transformed by electroporation into *E. coli* strain KC8 (Clontech KC8 electrocompetent cells, cat. # C2023-1), and the cells were selected on ampicillin-containing plates in the absence of either tryptophane (selection for the bait plasmid) or leucine (selection for the brain library plasmid). DNA for both plasmids was prepared and sequenced by di-deoxynucleotide chain termination method. The identity of the bait cDNA insert was confirmed and the cDNA insert from the brain library plasmid was identified using BLAST program against public

nucleotides and protein databases. Plasmids from the brain library (preys) were then individually transformed into yeast cells together with a plasmid driving the synthesis of lamin fused to the Gal4 DNA binding domain. Clones that gave a positive signal after β-galactosidase assay were considered false-positives and discarded. Plasmids for the remaining clones were transformed into yeast cells together with plasmid for the original bait. Clones that gave a positive signal after β-galactosidase assay were considered true positives.

EXAMPLE 2

Identification of AKT1/FNTA Interaction

[0065] A yeast two-hybrid system as described in Example 1 using amino acids 1-150 of Akt1 (GenBank (GB) accession no. M63167) as bait was performed. One clone that was identified by this procedure included amino acids 189-328 of FNTA (GB accession no. L00634).

EXAMPLES 3-11

Identification of Protein-Protein Interactions

[0066] A yeast two-hybrid system as described in Example 1 using amino acids of the bait as set forth in Table 11 was performed. The clone that was identified by this procedure for each bait is set forth in Table 11 as the prey. The "AA" refers to the amino acids of the bait or prey. The "NUC" refers to the nucleotides of the bait or prey. The Accession numbers refer to GB: GenBank accession numbers.

TABLE 11

Ex.	BAIT	ACCESSION	COORDINATES	PREY	ACCESSION	COORDINATES
3	Akt1	GB: M63167	AA: 1-150	TPRD	GB: D84294	AA 1058-1189
4	Akt1	GB: M63167	AA 1-118	KIAA0728	GB: AB018271	AA 254-469
5	Akt1	GB: M63167	AA 1-109	PPL	GB: NM_002705	AA 1548-1756
6	Akt1	GB: M63167	AA 1-118	Golgin-84	GB: NM_005113	AA 609-731
7	Akt2 p55	GB: M95936	AA 1-108	CLIC1	GB: X87689	AA 51-210
8	Akt2 p55	GB: M95936	AA 1-108	AKR7A2	GB: AF026947	AA 82-330
9	Akt2 p55	GB: M95936	AA 1-152	TPRD	GB: L07597	AA 1058-1189
10	p90RSK	GB: L07597	AA 600-736	KIAA0728	GB: AB018271	AA 45-469
11	p90RSK	GB: L07597	AA 418-675	UNR	GB: AB020692	AA 110-528

EXAMPLE 12

Generation of Polyclonal Antibody Against Protein Complexes

[0067] As shown above, Akt1 interacts with FNTA to form a complex. A complex of the two proteins is prepared, e.g., by mixing purified preparations of each of the two proteins. If desired, the protein complex can be stabilized by cross-linking the proteins in the complex, by methods known to those of skill in the art. The protein complex is used to immunize rabbits and mice using a procedure similar to that described by Harlow et al. (1988). This procedure has been shown to generate Abs against various other proteins (for example, see Kraemer et al., 1993).

[0068] Briefly, purified protein complex is used as immunogen in rabbits. Rabbits are immunized with 100 μg of the protein in complete Freund's adjuvant and boosted twice in three-week intervals, first with 100 μg of immunogen in incomplete Freund's adjuvant, and followed by 100 μg of immunogen in PBS. Antibody-containing serum is collected two weeks thereafter. The antisera is preadsorbed with Akt1 and FNTA, such that the remaining antisera comprises antibodies which bind conformational epitopes, i.e., complex-specific epitopes, present on the Akt1-FNTA complex but not on the monomers.

[0069] Polyclonal antibodies against each of the complexes set forth in Tables 1-10 are prepared in a similar manner by mixing the specified proteins together, immunizing an animal and isolating antibodies specific for the protein complex, but not for the individual proteins.

EXAMPLE 13

Generation of Monoclonal Antibodies Specific for Protein Complexes

[0070] Monoclonal antibodies are generated according to the following protocol. Mice are immunized with immunogen comprising Akt1/FNTA complexes conjugated to keyhole limpet hemocyanin using glutaraldehyde or EDC as is well known in the art. The complexes can be prepared as described in Example 12, and may also be stabilized by cross-linking. The immunogen is mixed with an adjuvant. Each mouse receives four injections of 10 to 100 μg of immunogen, and after the fourth injection blood samples are taken from the mice to determine if the serum contains antibody to the immunogen. Serum titer is determined by ELISA or RIA. Mice with sera indicating the presence of antibody to the immunogen are selected for hybridoma production.

[0071] Spleens are removed from immune mice and a single-cell suspension is prepared (Harlow et al., 1988). Cell fusions are performed essentially as described by Kohler et al. (1975). Briefly, P3.65.3 myeloma cells (American Type Culture Collection, Rockville, MD) or NS-1

myeloma cells are fused with immune spleen cells using polyethylene glycol as described by Harlow et al. (1988). Cells are plated at a density of $2x10^5$ cells/well in 96-well tissue culture plates. Individual wells are examined for growth, and the supernatants of wells with growth are tested for the presence of Akt1/FNTA complex-specific antibodies by ELISA or RIA using Akt1/FNTA complex as target protein. Cells in positive wells are expanded and subcloned to establish and confirm monoclonality.

[0072] Clones with the desired specificities are expanded and grown as ascites in mice or in a hollow fiber system to produce sufficient quantities of antibodies for characterization and assay development. Antibodies are tested for binding to Akt1 alone or to FNTA alone, to determine which are specific for the Akt1/FNTA complex as opposed to those that bind to the individual proteins.

[0073] Monoclonal antibodies against each of the complexes set forth in Tables 1-10 are prepared in a similar manner by mixing the specified proteins together, immunizing an animal, fusing spleen cells with myeloma cells and isolating clones which produce antibodies specific for the protein complex, but not for the individual proteins.

EXAMPLE 14

In vitro Identification of Modulators for Protein-Protein Interactions

[0074] The present invention is useful in screening for agents that modulate the interaction of Akt1 and FNTA. The knowledge that Akt1 and FNTA form a complex is useful in designing such assays. Candidate agents are screened by mixing Akt1 and FNTA (a) in the presence of a candidate agent, and (b) in the absence of the candidate agent. The amount of complex formed is measured for each sample. An agent modulates the interaction of Akt1 and FNTA if the amount of complex formed in the presence of the agent is greater than (promoting the interaction), or less than (inhibiting the interaction) the amount of complex formed in the absence of the agent. The amount of complex is measured by a binding assay, which shows the formation of the complex, or by using antibodies immunoreactive to the complex.

[0075] Briefly, a binding assay is performed in which immobilized Akt1 is used to bind labeled FNTA. The labeled FNTA is contacted with the immobilized Akt1 under aqueous conditions that permit specific binding of the two proteins to form a Akt1/FNTA complex in the absence of an added test agent. Particular aqueous conditions may be selected according to conventional methods. Any reaction condition can be used as long as specific binding of Akt1/FNTA occurs in the control reaction. A parallel binding assay is performed in which the test

agent is added to the reaction mixture. The amount of labeled FNTA bound to the immobilized Akt1 is determined for the reactions in the absence or presence of the test agent. If the amount of bound, labeled FNTA in the presence of the test agent is different than the amount of bound labeled FNTA in the absence of the test agent, the test agent is a modulator of the interaction of Akt1 and FNTA.

[0076] Candidate agents for modulating the interaction of each of the protein complexes set forth in Tables 1-10 are screened *in vitro* in a similar manner.

EXAMPLE 15

In vivo Identification of Modulators for Protein-Protein Interactions

[0077] In addition to the *in vitro* method described in Example 14, an *in vivo* assay can also be used to screen for agents which modulate the interaction of Akt1 and FNTA. Briefly, a yeast two-hybrid system is used in which the yeast cells express (1) a first fusion protein comprising Akt1 or a fragment thereof and a first transcriptional regulatory protein sequence, e.g., GAL4 activation domain, (2) a second fusion protein comprising FNTA or a fragment thereof and a second transcriptional regulatory protein sequence, e.g., GAL4 DNA-binding domain, and (3) a reporter gene, e.g., β-galactosidase, which is transcribed when an intermolecular complex comprising the first fusion protein and the second fusion protein is formed. Parallel reactions are performed in the absence of a test agent as the control and in the presence of the test agent. A functional Akt1/FNTA complex is detected by detecting the amount of reporter gene expressed. If the amount of reporter gene expression in the presence of the test agent is different than the amount of reporter gene expression in the absence of the test agent, the test agent is a modulator of the interaction of Akt1 and FNTA.

[0078] Candidate agents for modulating the interaction of each of the protein complexes set forth in Tables 1-10 are screened *in vivo* in a similar manner.

[0079] While the invention has been disclosed in this patent application by reference to the details of preferred embodiments of the invention, it is to be understood that the disclosure is intended in an illustrative rather than in a limiting sense, as it is contemplated that modifications will readily occur to those skilled in the art, within the spirit of the invention and the scope of the appended claims.

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